METHODS

The present invention relates to a novel detection system comprising novel primers and an integrated signalling system. The system is used in the detection of target nucleic acid sequences.

Available methods for the amplification and detection of target nucleic acid sequences include use of the polymerase chain reaction (PCR), for example as described in United States patents nos. 4683195 and 4683202.

A significant improvement on the above amplification and detection methods is the Amplification Refractory Mutation System (ARMS) as claimed in our European Patent no. 0 332 435 (Zeneca Limited) and corresponding US Patent No. 5595890.

Convenient probe based detection systems include Taqman (as disclosed in US patents nos. 5210015 and 5487972) and Molecular Beacons (as disclosed in WO-95/13399). In Taqman a probe molecule comprising fluorophore/quencher species hybridises to PCR amplification products and is digested by the 5'-3' exonuclease activity of a polymerase. This leads to release of unquenched fluorophore and a corresponding detectable signal. In Molecular Beacons a probe molecule having a stem-loop structure keeping fluorophore and quencher species in close proximity opens out upon binding to its complementary target whereupon the fluorophore becomes unquenched leading to a detectable signal.

Nazarenko et al (NAR 1997, 25, 2516-2521) disclose so called "Sunrise" primers. These are primers which form hairpin loops at their 5' ends to bring a fluorophore and quencher pair together, thus ensuring low fluorescence. When these primers have been incorporated into a PCR product, the tails become double stranded and the hairpin is unravelled causing the fluorescence to increase. However signal generation is not amplicon dependent, any double stranded amplicon (including primer dimers) can incorporate the Sunrise primer and thus generate a spurious signal.

US-A-5573906 (Bannwarth et al) describe a process using a 5' labelled primer containing a self-complementary sequence in an amplification or extension process together with a subsequent detection step using a 3' labelled probe for the amplified or extended region. The labels may be close together in space after hybridising the probe close to the

short piece of double-stranded DNA resulting from backfolding of the self-complementary region of the primer incorporated into the amplified or extended product.

However, the levels of sequence specificity and detection sensitivity, as well as speed of signal appearance, achievable using the above amplification and detection methods are limited. Therefore the need still exists for further improved diagnostic methods.

We have now devised a novel detection system using a tailed primer and an integrated signalling system. The primer has a template binding region and a tail comprising a linker and a target binding region. In use the target binding region in the tail hybridises to complementary sequence in an extension product of the primer. This target specific hybridisation event is coupled to a signalling system wherein hybridisation leads to a detectable change.

Therefore in a first aspect of the present invention we provide a method for the detection of a target nucleic acid, which method comprises contacting template nucleic acid from a sample with (i) a signalling system and (ii) a tailed nucleic acid primer having a template binding region and the tail comprising a linker and a target binding region, in the presence of appropriate nucleoside triphosphates and an agent for polymerisation thereof, under conditions such that the template binding region of the primer will hybridise to a complementary sequence in the template nucleic acid and be extended to form a primer extension product, separating any such product from the template whereupon the target binding region in the tail of the primer will hybridise to a sequence in the primer extension product corresponding to the target nucleic acid, and wherein any such target specific hybridisation causes a detectable change in the signalling system, such that the presence or absence of the target nucleic acid in the sample is detected by reference to the presence or absence of a detectable change in the signalling system.

The detection method of the invention has a number of significant advantages. These include the following. Only a single primer/detector species is required. This means simplicity and provides enhanced specificity based on the ready availability of the target binding region for hybridisation with the primer extension product. The newly synthesised primer extension product is the target species so the output signal obtainable is directly related to amount of extended primer. It is not dependent on additional hybridisation events or enzymatic steps (such as TaqMan cleavage). Intra- and inter-strand competition for the probe

site is limited so probe design becomes simplified. We have found that probes which fail to bind under standard assay conditions in separate probe format work well in our invention. The invention also allows homogeneous assay formats to be readily devised. A still further advantage is that, as the interaction is unimolecular, the signalling reaction is very rapid, permitting increased cycling rates. This is a significant feature for assay designs.

Wilton et al (Human Mutation, 1998, 11, 252-258) disclose an analytical method termed Snapback Single Strand Conformation Polymorphism (SSCP). This involves the use of a tailed primer to introduce a secondary structure in a single strand of an amplicon. The primers consist of standard 3' ends with short tails on the 5' end. These tails are complementary to an internal region of the amplicon at some distance from the primer and can be used to probe the conformation of the single strands formed after heating and cooling. The conformational changes introduced by a mutation at the probe complementary site are detected by migration rate changeson a polyacylamide gel stained with silver. However there is no anticipation of the features or advantages of the present invention.

In the detection method of the invention, primer extension may be repeated one or more times such as up to 5, up to 10, up to 15, up to 20, up to 30,up to 40, up to 50 or more times. Conveniently, the novel primer of the invention is used as an amplification primer in an amplification system such as the polymerase chain reaction (PCR). In which case the target binding region and the tail region are advantageously arranged such that the tail region remains single stranded, ie. uncopied. Thus the tail region is non-amplifiable in the PCR amplification products. This facet of primer design is claimed in our European Patent No. 0 416 817 (Zeneca Limited) and corresponding US Patent No. 5525494. Conveniently the linker comprises a blocking moiety which prevents polymerase mediated chain extension on the primer template. A preferred blocking moiety is a hexethylene glycol (HEG) monomer. Alternatively the primer tail comprises material such as 2-O-alkyl RNA which will not permit polymerase mediated replication of a complementary strand. Alternatively the tail comprises nucleic acid placed 5'-3' at the 5' terminus of the primer ie. the two sequences are placed "back to back", it will be appreciated that in this embodiment the 5'-3' nucleic acid of the tail serves both as the linker and the target binding region. A separate and distinct linker moiety is not essential.